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Root Carbon Dioxide Fixation by Phosphorus-Deficient *Lupinus albus*[1]

Contribution to Organic Acid Exudation by Proteoid Roots

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When white lupin (*Lupinus albus* L.) is subjected to P deficiency lateral root development is altered and densely clustered, tertiary lateral roots (proteoid roots) are initiated. These proteoid roots exude large amounts of citrate, which increases P solubilization. In the current study plants were grown with either 1 mM P (+P-treated) or without P (−P-treated). Shoots or roots of intact plants from both P treatments were labeled independently with $^{14}CO_2$ to compare the relative contribution of C fixed in each with the C exuded from roots as citrate and other organic acids. About 25-fold more acid-stable ^{14}C, primarily in citrate and malate, was recovered in exudates from the roots of −P-treated plants compared with +P-treated plants. The rate of in vivo C fixation in roots was about 4-fold higher in −P-treated plants than in +P-treated plants. Evidence from labeling intact shoots or roots indicates that synthesis of citrate exuded by −P-treated roots is directly related to nonphotosynthetic C fixation in roots. C fixed in roots of −P-treated plants contributed about 25 and 34% of the C exuded as citrate and malate, respectively. Nonphotosynthetic C fixation in white lupin roots is an integral component in the exudation of large amounts of citrate and malate, thus increasing the P available to the plant.

P deficiency in white lupin (*Lupinus albus* L.) enhances the development of cluster or proteoid root morphology (Gardner et al., 1981; Marschner et al., 1986; Dinkelaker et al., 1989; Johnson et al., 1994; Braum, 1995). Proteoid roots are the specific site of citrate excretion (Marschner et al., 1987; Dinkelaker et al., 1989). Citrate excreted by P-deficient white lupin can represent as much as 11% (Gardner et al., 1983) to 23% (Dinkelaker et al., 1989) of the total plant dry weight, depending on plant age and the severity of the P stress.

Carboxylic acids, particularly citric acid, play a role in the acquisition of nutrients, especially P. Exudation of citrate into the rhizosphere is thought to increase the availability of P to plants by mobilizing sparingly soluble Fe and Al phosphates (Gardner et al., 1982, 1983) or Ca-P (Dinkelaker et al., 1989). Organic acid exudation by rape (*Brassica napus* L.) plants was shown to be an effective strategy to increase P uptake from rock phosphate (Hoffland, 1992). The complexing action of the citrate anion and the dissolution properties of the protons released from citric acid as it equilibrates with the soil solution can mobilize P from soil with a large Ca-P fraction (Jones and Darrah, 1994). Citrate acts via several mechanisms to mobilize soil P, affecting both inorganic and organic P fractions by (a) anion exchange; (b) chelation of bridging metal cations from organic matter in the soil; and (c) suppressing readsorption and precipitation of inorganic P (Gerke et al., 1994; Braum, 1995).

CO_2 fixation by root systems is generally considered negligible (Farmer and Adams, 1991); however, C fixed in roots may be important for root C metabolism (Vuorinen et al., 1992). A significant amount of the CO_2 fixed in roots is transported to shoots (Maxwell et al., 1984; Miller et al., 1990; Cramer et al., 1993) and may result in increased biomass production (Vapaavuori and Pelkonen, 1985). In legumes, C fixed in effective root nodules was estimated to provide 25 to 30% of the C required for N assimilation (Vance et al., 1983; Maxwell et al., 1984). Similarly, C fixed in the roots of non-legumes provides an important source of C for NH_4^+ assimilation (Ikeda et al., 1992; Cramer et al., 1993). A portion of the C used for citrate synthesis in proteoid roots of white lupin can be derived from nonphotosynthetic C fixation via PEPC (Johnson et al., 1994).

In prior work (Johnson et al., 1994), we proposed that increased PEPC (EC 4.1.1.31) in the proteoid roots of P-deficient white lupin may buffer against losses of photo-

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Abbreviations: CS, citrate synthase; dabsyl-Cl, (dimethylamino)-azobenzenesulfonyl chloride; DAE, days after emergence; LSC, liquid scintillation counter; MDH, malate dehydrogenase; OAA, oxaloacetic acid; PEPC, PEP carboxylase; SAX, strong anion-exchange chromatography; SCX, strong cation-exchange chromatography.

synthetic C during citrate excretion from roots. Other studies also have suggested a role for PEPC in organic acid synthesis and root exudation in nutrient-deficient plants (Landsberg, 1986; Hoffland et al., 1992; Pilbeam et al., 1993). Shoot PEPC may contribute to increased citrate synthesis in P-deficient rape plants (Hoffland et al., 1990, 1992). Hoffland et al. (1992) hypothesized that malic acid (the dominant root exudate of rape) was synthesized within the excreting root segment by carboxylation of PEP by PEPC. Increased activity of PEPC in both the leaves and roots of P-deficient tomato (*Lycopersicon esculentum* L.) was hypothesized to contribute to cation balance and improve P acquisition by increasing the rates of either proton or organic acid excretion (Pilbeam et al., 1993). In response to Fe deficiency, increased PEPC activity was reported in the zone of maximum proton efflux of excised roots of pepper (*Capsicum annuum* L.) (Landsberg, 1986). When tomato roots were labeled with $^{14}CO_2$, the amount of ^{14}C in organic acids from Fe-deficient plants increased compared with that from Fe-adequate plants (Miller et al., 1990).

The relative contribution of shoot- and root-fixed C to exuded acids is not known. In this study, intact root or shoot systems were labeled independently with $^{14}CO_2$ and the distribution of ^{14}C-label among primary metabolites in plant organs and root exudates was determined. The partitioning of fixed ^{14}C between root exudates and plant organs was used to estimate the relative contribution of C fixed in shoots or roots to C exuded as citrate.

MATERIALS AND METHODS

Lupinus albus L. var Ultra plants were grown in a growth chamber (Conviron, model no. E15, Controlled Environments, Pembina, ND) at 20/15°C and 16-/8-h day/night cycles, 300 μmol photons m^{-2} s^{-1} at shoot height and 70% RH. Three surface-sterilized seeds were planted in each pot containing 5 kg of 1.0-mm-diameter silica sand. After emergence, plants were thinned to one plant per pot and grown for an additional 14 d. Pots were watered every 2 d with 1.0 L of the appropriate nutrient solution (Johnson et al., 1994). The nutrient solutions differed in P concentration: the +P treatment group received 0.5 mM $Ca(H_2PO_4)_2$, whereas the −P treatment group received 0.5 mM $CaSO_4$ and no P. The pH of the nutrient solution was adjusted to 6.0 for both treatments.

^{14}C Labeling Conditions

Plants were placed in an exhaust hood 24 h before labeling to allow them to acclimate to the new temperature and lighting conditions. In the hood the temperature was approximately 26°C at shoot height and RH was ambient. The day/night cycle of 16/8 h was maintained. The light source in the hood was a high-pressure Na growth light mounted about 1 m above the plants, providing 700 μmol photons m^{-2} s^{-1} at shoot height. Excess nutrient solution was removed from each pot with a slight suction (0.03–0.05 MPa) to facilitate gas circulation and exchange around the roots just before labeling. An aliquot of this solution was retained as time-zero exudate.

Entire shoot or root systems were labeled independently, and are referred to as "shoot-labeled" and "root-labeled" plants. Labeling occurred at midmorning, after the plants had been exposed to light for about 4 to 6 h. One +P-treated and one −P-treated plant were labeled in series at 14 DAE. The root and shoot systems were separated with a sheet of polyvinyl chloride cut to the inner diameter of the lower pot and sealed around the edges with window caulking. The polyvinyl chloride cover had a narrow slot for the shoot, which was also sealed. A 4-L clear acrylic chamber was positioned and sealed over the shoot to create the shoot-labeling chamber. Chamber volume was 4200 cm^3 for root labeling and 4400 cm^3 for shoot labeling. A pneumatic pump was used to recirculate the air within the closed labeling chambers. Flow meters were used to verify that there were no leaks before or during $^{14}CO_2$ generation.

Roots were exposed to 3.7 MBq and shoots to 0.37 MBq $^{14}CO_2$ (2.07 MBq μmol^{-1} $NaH^{14}CO_3$) for 60 min. The $^{14}CO_2$ was generated by adding 2 M HCl to $NaH^{14}CO_3$ solution with a syringe. At the end of the pulse, fresh air was drawn through the chambers with a vacuum (0.03–0.05 MPa) for about 10 min (at least 15 void volumes). The unincorporated radioactivity was trapped with two 1-M NaOH traps and a charcoal trap, arranged in series. The shoots were uncovered at this time and 100 mL of nutrient solution was added to replace the nutrient solution removed before the pulse.

The concentration of CO_2 was monitored every 10 min during the pulse by a gas chromatograph equipped with a thermal conductivity detector (Carle GC-8700, EG&G Chandler Engineering, Tulsa, OK). The carrier gas was He. A 5.0 ± 0.1% CO_2 standard was used for calibration. CO_2 concentration was maintained near ambient levels during the shoot labeling by injecting 50 mL of compressed CO_2 (5%) at 10- to 15-min intervals. Root respiration averaged 0.46 nmol CO_2 min^{-1} during root labeling, which increased the CO_2 concentration in the chamber to about twice that of ambient by the end of the pulse. The average CO_2 concentration during root or shoot labeling was used to determine the specific radioactivity in the respective chambers. Specific radioactivity averaged 15 Bq nmol^{-1} CO_2 generated in the root-labeling chambers, and 2.6 Bq nmol^{-1} CO_2 in the shoot-labeling chambers.

Assumptions and Sample Calculations

To estimate the contribution of C fixed in roots or shoots to root exudates, the specific radioactivity (Bq nmol^{-1} $^{14}CO_2$) in the labeling chamber was used to calculate total C fixed. Calculations were based on the following assumptions (Maxwell, 1982; Maxwell et al., 1984): (a) all radioactivity in plant extracts and root exudates is either from C fixed in the root or in the shoot, depending on the site of labeling; (b) for the purpose of calculation, the CO_2 concentration surrounding the roots or shoots is the average of the five CO_2 measurements taken during the pulse; (c) the ideal gas law holds; (d) all $NaH^{14}CO_3$ in the reaction flask was completely converted to $H^{14}CO_3$ and then to $^{14}CO_2$ when acidified; and (e) the amount of total C exuded by plants was not altered by labeling treatments.

Specific radioactivity in the labeling chamber was calculated as follows:

$$\frac{\text{nmol } {}^{14}CO_2 \text{ generated}}{\text{nmol } CO_2 \text{ in chamber}} \times \frac{1 \text{ nmol } NaH{}^{14}CO_3}{\text{nmol } {}^{14}CO_2 \text{ generated}} \times \frac{Bq}{\text{nmol } NaH{}^{14}CO_3} = \frac{Bq}{\text{nmol } CO_2 \text{ in chamber}}. \quad (1)$$

Calculation of the CO_2 fixation rate was based on total Bq (acid stable recovered in the plant and in the corresponding exudate) that was fixed in the 60-min pulse period. Only one plant was grown per pot; therefore, the total acid-stable radioactivity represents the total amount of ^{14}C incorporated per plant during the 60-min chase:

$$\frac{\text{total Bq incorporated in 60 min}}{} \times \frac{1}{\text{g fresh wt}} \times \frac{\text{g fresh wt}}{\text{g fresh wt root (or shoot)}} \times \frac{\text{nmol } CO_2 \text{ in chamber}}{Bq} = \text{nmol } CO_2 \text{ min}^{-1} \text{ g}^{-1}. \quad (2)$$

The amount of C fixed during the 60-min pulse recovered in crude exudate was calculated as follows:

$$\frac{Bq}{\text{L exudate}} \times \frac{\text{nmol C in chamber}}{Bq} = \text{nmol C L}^{-1} \text{ exudate}. \quad (3)$$

The amount of C fixed during the 60-min pulse recovered in citrate or malate was calculated as follows:

$$\frac{Bq}{\mu\text{mol citrate (or malate)}} \times \frac{\text{nmol C in chamber}}{Bq} \times \frac{\mu\text{mol citrate (or malate)}}{\text{L exudate}} = \text{nmol C L}^{-1} \text{ exudate (in citrate or malate)}. \quad (4)$$

Assuming that the sum of ^{14}C exuded from root-labeled and shoot-labeled plants is proportional to the total C exuded from the roots, the ratio of C exuded from root-labeled plants to the sum of C exuded from both root-labeled and shoot-labeled plants would be the percentage contribution of CO_2 fixed in roots:

$$\frac{\text{C fixed in roots}}{\text{C fixed in root + C fixed in shoot}} \times 100 = \% \text{ contribution of root fixed C}. \quad (5)$$

Exudate Collection

After labeling plants were kept at 16-/8-h day/night under the high-pressure Na light in the fume hood for a 22-h chase period. Preliminary experiments demonstrated that the majority of radioactivity destined for exudation was exuded by 3 h into the chase. Root exudates were collected after 3 h and just prior to plant harvest at 22 h into the chase; results from both exudate collections were summed after HPLC. The plants were watered with 1000 mL of nutrient solution and the eluted solution collected from the bottom of the chamber. The eluted nutrient solution contained water-soluble root exudates and is referred to as the "root exudate." A slight suction (0.03–0.05 MPa) was applied to ensure that no radioactive solution leaked from the bottom of the pots. A 200-μL aliquot was treated with HCl and acid-stable radioactivity in the crude root exudate was determined with a LSC (model LS8600, Beckman). A 200-mL aliquot, from which a 25-mL aliquot of well-mixed root exudate was concentrated to approximately 10 mL in an auto evaporator (Savant Speedvac, Savant Instruments, Farmington, NY), was retained and stored in the freezer. Samples were stored at −20°C.

Plant Harvest

The roots were washed gently to remove adhering sand, separated into normal and proteoid roots, and weighed. Proteoid roots were defined as those secondary laterals with 10 or more tertiary roots per centimeter. It was demonstrated that exudation of citrate is confined to the proteoid root zone (Marschner et al., 1987; Dinkelaker et al., 1989). Therefore, only the portion of the secondary root displaying clustered tertiary roots was collected as a proteoid root segment, excluding the most basal and apical portions. Normal root segments were comparable portions of secondary roots with no or few (less than five) tertiary roots per centimeter, also excluding the most basal and apical portions. About 0.75 g of each root type or leaf was used for extracting metabolites. Total shoot mass was determined and the youngest fully expanded leaves were used for the extraction. Plant material was kept on ice during harvest. Grinding was conducted inside a disposable plastic glove bag (Cole-Parmer Instruments, Niles, IL) on the bench top. Plant tissue was placed in a mortar, frozen with liquid N_2, ground to a fine powder, extracted with 10 mL of 50% ethanol by boiling for 10 min in an 80°C water bath, and centrifuged at 10,000*g* for 20 min (Christeller et al., 1977; Maxwell et al., 1984). The supernatant was filtered through a surety filter column (Evergreen Scientific International, Los Angeles, CA). Hydrophobic compounds were removed with a chloroform separation (2:1, supernatant:chloroform). An aliquot of the water-soluble layer was treated with HCl, and the acid-stable radioactivity was determined with a LSC.

Ion-Exchange Chromatography

The acidic fraction of each sample was separated from the basic and neutral fractions using SAX (SAX Bond Elute columns, capacity 2.8 mL 500 mg^{-1}; Analytichem International, Varian, Harbor City, CA). The columns were preconditioned with 2 mL of 100% methanol followed by 4 mL of 50% ethanol. The sample (organ extract or root exudate) was loaded onto the column, washed with 2 mL of 50% ethanol (neutral plus basic fraction), and then eluted with 4 mL of 2% HCl in methanol (acidic fraction). Radioactivity was determined with a LSC.

After anion exchange, the neutral plus basic fraction was dried and the residue was resuspended in 1.0 mL of 50% ethanol and acidified to pH 2. SCX (SCX Bond Elute col-

umns, capacity 2.8 mL 500 mg^{-1}; Analytichem International) was used to separate neutral and basic fractions. The SCX columns were conditioned with 2 mL of 100% methanol followed by 2 mL of 2% HCl in absolute ethanol. The entire sample was loaded, washed with 2 mL of 50% ethanol (neutral fraction), and eluted with 10 mL of 28% acetonitrile and 2% HCl in methanol (basic fraction). The radioactivity in each fraction was determined with a LSC.

HPLC for Organic and Amino Acids

The acidic fraction was dried and the residue was resuspended in 0.008 N H_2SO_4, syringe filtered (0.45 μm), and stored at −20°C until HPLC analysis. The individual organic acids from plant organ extracts and root exudates were separated by HPLC (model SP800 pump and SP4290 integrator, Spectra-Physic, San Jose, CA) equipped with an Aminex HPX-300 × 7.8 mm-column (Bio-Rad) and an organic acid guard column (Bio-Rad). The eluant was 0.008 N H_2SO_4 with a flow rate of 0.6 mL min^{-1} at 25°C. Individual acids were detected at 210 nm on a UV/VIS detector (Spectroflow 757, ABI Analytical, Kratos Division, Ramsey, NJ) and collected with a fraction collector (model FC203, Gilson, Middleton, WI) as described by Johnson et al. (1994). Average recovery after ion exchange and HPLC of organic acids was 96% for citrate, 66% for α-ketoglutarate, 98% for malate, 50% for succinate, and 81% for fumarate, determined from external standard solutions. Total radioactivity was determined for each peak with a LSC.

Root exudates from +P-treated plants had very low concentrations of organic acids, frequently below the detection limit of the detector and/or the fraction collector, resulting in few data points for citrate and malate concentration. In addition, the total amount of radioactivity exuded from the roots of +P-treated plants was close to background levels, thus increasing the error and resulting in radioactivity below the detection limit for individual organic acids. Therefore, only the radioactivity of individual organic acids exuded from the roots of −P-treated plants is reported.

A 1-mL aliquot was taken from the extracts prior to ion-exchange chromatography to prevent the conversion of Asn and Gln to Asp and Glu under the acidic conditions used. A 20-μL aliquot was derivatized with dabsyl-Cl (Knecht and Chang, 1986). A standard of mixed amino acids was included in each dabsylation set to correct for variability in derivatization. After derivatization, samples were stored at 4°C for no more than 48 h before HPLC injection.

Individual amino acids were separated with a 25 cm × 4.6 cm column (model S5 ODS2, Spherisorb, Clwyd, UK) with a model ODS-5S guard column using a mixture of solution A, 25 mM sodium acetate, pH 6.5, with 0.4% dimethylformamide (degassed with helium); and solution B, acetonitrile. The gradient was 15 to 40% B for 20 min, 40 to 70% B from 20 to 32 min, kept at 70% B from 32 to 34 min, then returned to 15% B from 34 to 36 min (modified from Knecht and Chang [1986]). The column temperature was 40°C and the flow rate was 1 mL min^{-1}. The amino acids were detected at A_{436} on a UV/VIS detector (Spectroflow 757, ABI). External standards were used to determine the retention time and retention factors for derivatized Asp, Glu, Asn, Gln, Ser, Thr, Gly, Ala, Pro, Val, Met, Arg, Iso, Leu, Phe, Cys, Lys, His, and Tyr. These values were used to identify sample amino acid peaks and concentrations. Preliminary analysis indicated that the radioactivity in individual amino acids from the crude samples was below the detection limit of the LSC; therefore, individual amino acid peaks were not collected.

In two replicates the dabsyl-Cl background and Asn peaks could not be resolved. This may have been due to aging of the column or to fluctuations in flow rate. The presence of Asn co-eluted with the dabsyl-Cl was confirmed in both replicates by TLC separation for amino acids as described by Platt and Rand (1979), but could not be quantified.

Enzyme Activity

Plants used for enzyme activity studies were grown to 14 DAE under the conditions described above for the ^{14}C labeling experiments. About 0.5 g of normal roots, proteoid roots, or leaves (youngest fully expanded) were used for the enzyme extraction. The fresh material was frozen with liquid N_2, ground to a fine powder, and then homogenized with Tris-Cl buffer (0.25 mM, pH 8.0, with 0.5 M Suc, and 0.001 M DTT) as previously described (Johnson et al., 1994). After homogenization 0.1 M PMSF in isopropanol was added (10 μL mL^{-1} buffer) to inhibit protease activity. The homogenate was centrifuged at 14,000*g* for 10 min, and the supernatant was used to determine PEPC activity spectrophotometrically by monitoring the disappearance of NADH at A_{340} in a MDH-coupled assay for 2 min (Vance et al., 1983). Total protein in the supernatant was determined with a bicinchoninic acid protein assay reagent kit (Pierce) after acetone precipitation (Johnson et al., 1994).

Statistical Analysis

A general linear model for analysis of variance was used to determine statistical differences. The labeling experiments were a split-split plot design with site of label the main effect, P treatment as the subplot effect, and organ type or time of collection as the subsubplot (Gomez and Gomez, 1984). Five separate labeling events were treated as replicates. The LSD values for the interaction terms used weighted SD and time values (Gomez and Gomez, 1984). In the event of one missing data point in a set, an estimate of missing-data technique was applied (Gomez and Gomez, 1984).

The recovery of radioactivity in the ion-exchange fractions from one replication of root exudate was very low (more than 2 SD from the mean), although at the time of collection these samples had a high amount of radioactivity. This loss presumably was due to microbial degradation after the samples were inadvertently left at room temperature. Such degradation would lower acid-stable radioactivity as the metabolites were consumed and respired. Therefore, only four replications were used in the statistical analysis for ion exchange of root exudates and HPLC analysis of organic acids from root

exudates. Only the three replicates in which Asn was resolved from dabsyl-Cl were included in the statistical analysis of amino acid concentration.

RESULTS

Distribution of Label Retained in the Plant and in Root Exudates

The −P-treated plants exuded 13- to 33-fold more acid-stable radioactivity than the corresponding +P-treated plants (Table I). The majority of the acid-stable radioactivity (66%) was recovered in root exudates from −P-treated plants when roots were labeled; 32% was recovered when shoots were labeled (Table I). By comparison, more than 90% of the acid-stable fixed ^{14}C was retained in the +P-treated plants, regardless of the labeling site. When roots were labeled the −P-treated plants incorporated about 3-fold more acid-stable radioactivity than the +P-treated plants. The amount of acid-stable radioactivity incorporated was similar between P treatments when shoots were labeled. Acid-stable radioactivity does not include respired $^{14}CO_2$, which may represent 5 to 45% of photosynthetic C (Whipps and Lynch, 1983; Whipps, 1990; Shephard and Davies, 1993).

Distribution of Radioactivity among Ion-Exchange Fractions from Root Exudates

Of the substantial amount of radioactivity exuded from −P-treated plants (Table I), 92% was recovered in organic acids, with most of the remaining radioactivity recovered in sugars, and only a trace in amino acids (Fig. 1). By comparison, of the small amount of acid-stable radioactivity exuded from +P-treated plants (Table I), 75% was in the acidic fraction (organic acids) and 17% in the neutral fraction (sugars), with the balance in the basic fraction (amino acids) (Fig. 1). Analysis of variance indicated that the P treatment accounted for all variability in the distribution of radioactivity among acidic, basic, and neutral fractions from root exudates (data not shown). Therefore, the data represent means averaged across the site of labeling (Fig. 1).

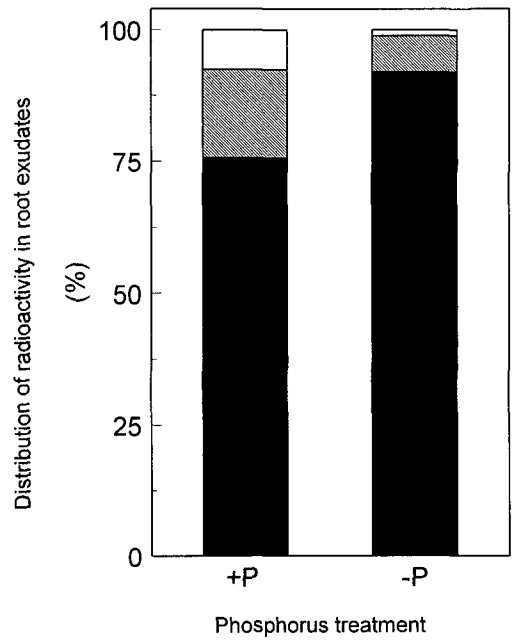

Figure 1. Distribution of ^{14}C radioactivity among acidic (black area, LSD = 7.5; organic acids), neutral (hatched area, LSD = 7.5; sugars), and basic (white area, LSD = 3.4; amino acids) fractions after ion-exchange chromatographic separation of *L. albus* root exudates. Plants were grown with either 1 mM P (+P-treated) or without P (−P-treated) for 14 DAE and shoots or roots were labeled independently with $^{14}CO_2$. Shoot-labeled plants were exposed to 0.37 MBq and root-labeled plants were exposed to 3.7 MBq $^{14}CO_2$ for 60 min followed by a 22-h chase. Exudates were collected as eluted nutrient solution at 3 and 22 h into the chase. All variability in the distribution of radioactivity was accounted for by P treatment; therefore, values are averaged over collection time and site of label, $n = 20$.

Distribution of Radioactivity among Ion-Exchange Fractions from Roots and Leaves

In normal and proteoid roots the majority of the radioactivity was recovered in the acidic fraction when the roots were labeled, but in the neutral fraction when shoots were labeled (Fig. 2). A similar pattern was observed in the leaves, with a larger percentage of radioactivity recovered

Table I. *Amount of ^{14}C radioactivity (in kBq) retained in whole plants or recovered in root exudate after labeling*

Plants were grown with either 1 mM P (+P treated) or without P (−P treated) for 14 DAE and shoots and roots were labeled independently with $^{14}CO_2$. Shoot-labeled plants were exposed to 0.37 MBq and roots to 3.7 MBq $^{14}CO_2$ for a 60-min pulse period followed by a 22-h chase. Exudates were collected as eluted nutrient solution at 3 and 22 h into the chase ($n = 5$).

Site of $^{14}CO_2$ Labeling	Treatment	Plant	Root Exudate	Total	Percentage in Root Exudates
			kBq		
Root	+P	14.8	0.9	15.7	5
Root	−P	17.0	33.1	50.0	66
Shoot	+P	34.3	1.0	35.2	3
Shoot	−P	29.3	13.3	42.6	32
Significance of labeling site		**[a]	**	NS	**
Significance of treatment		NS[b]	**	**	**
LSD for interactions ($\alpha = 0.05$)		NA[c]	4.6	12.4	11

[a] **, Significant at 0.01 level. [b] NS, Not significant at P = 0.01 level. [c] NA, LSD not applicable; interaction term was not significant.

in organic acids when roots were labeled compared with when shoots were labeled. There was a tendency for the −P-treated root-labeled organs to have more radioactivity in the amino acids compared with the other P treatments and labeling sites (Fig. 2). Radioactivity incorporated during photosynthesis would be incorporated initially into sugars that could be metabolized into organic acids or amino acids or be translocated to the roots. The majority of radioactivity was recovered in the organic acid fraction after incorporation of $^{14}CO_2$ by roots from several species supplied with different N sources: atmospheric N_2 (nodulated legumes), NO_3^-, or NH_4^+ (Christeller et al., 1977; Maxwell et al., 1984; Ikeda et al., 1992; Johnson et al., 1994). In contrast, a nearly equal amount of radioactivity was recovered in organic and amino acid fractions after incorporation of $^{14}CO_2$ by maize (*Zea mays* L.) roots supplied with NH_4^+ (Cramer et al., 1993).

Distribution and Radioactivity of Organic Acids in Root Exudates

Citrate and malate were the only organic acids recovered in root exudates after the 22-h chase (Table II). Sixteen-fold more citrate and malate was recovered in root exudates from −P-treated plants compared with +P-treated plants (averaged over the site of labeling). The root systems from −P-treated plants were 25% smaller than those from +P-treated plants (Table II). The exudation rate was estimated from the amount of citrate and malate recovered in the root exudates. The exudation rate of citrate and malate per unit weight of root was about 20-fold higher from −P-treated plants compared with +P-treated plants (Table II). The increased exudation rate per gram fresh weight indicates that differences in total citrate and malate exuded are not simply due to differences in total plant mass.

A total of 227 μmol of citrate and 156 μmol of malate were recovered from −P-treated root exudates during a 70-h interval, but only 11 μmol of citrate and 9 μmol of malate were recovered from +P-treated root exudates after the same interval. These values are based on 124 μmol of citrate and 96 μmol of malate recovered in exudates from −P-treated plants, and 6 μmol of citrate and 5 μmol of malate recovered in exudates from +P-treated plants that were collected prior to labeling (data not shown) and during the 22-h chase (Table II). This is equivalent to 3.3 and 1.7% of the total plant dry weight of −P-treated plants for citrate and malate, respectively, 20-fold more than for +P-treated plants. Plant dry weight assumes 85% plant moisture for average fresh weights of 10.4 g (+P-treated) and 8.4 g (−P-treated). When root exudates were allowed to accumulate for 48 h (collected just before labeling), a small amount of succinate was detected in some −P-treated exudate samples (data not shown).

The specific radioactivity of citrate and malate in root exudates from −P-treated plants was higher when roots were labeled than when shoots were labeled (Table II). In addition, citrate had a higher specific radioactivity than malate irrespective of the site of labeling, indicating that, in citrate, more C was derived from direct $^{14}CO_2$ fixation compared with malate. The low concentration of citrate, malate, and radioactivity in root exudates from the +P-treated plants prevented the determination of citrate- and malate-specific radioactivity in exudates.

Distribution and Specific Radioactivity of Organic Acids in Roots and Leaves

Citrate, malate, and succinate were the three primary acids detected in roots and leaves (Table III); fumarate and several unidentified acids were also detected by HPLC (data not shown). The concentration of citrate was higher in roots (normal and proteoid) compared with −P-treated leaves or any +P-treated organ (Table III). Malate and succinate concentrations were lower in roots than in leaves after both P treatments (Table III). Citrate from −P-treated normal and proteoid roots and +P-treated proteoid roots had a higher specific radioactivity than leaves from either treatment (Table III). In addition, the specific radioactivity of citrate was about 2-fold higher than that of malate in the roots of −P-treated plants. About 90% of the radioactivity in the organic acids was recovered in citrate, malate, and succinate, and the remaining radioactivity was recovered in fumarate and the unidentified peaks (data not shown).

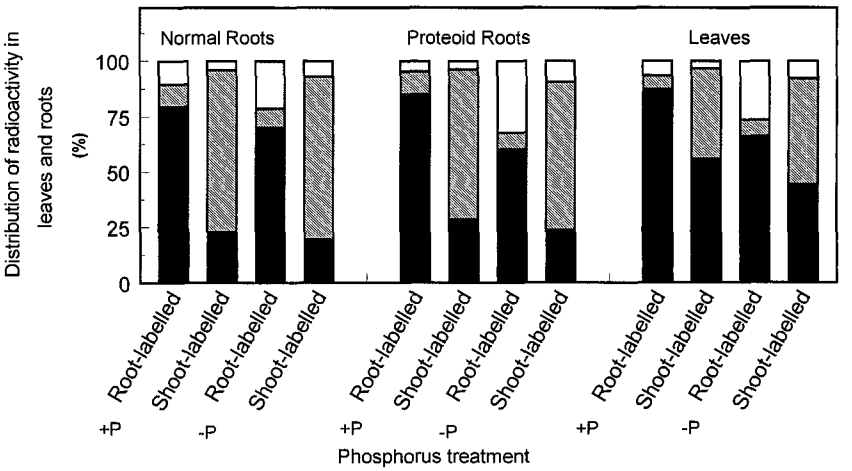

Figure 2. Distribution of ^{14}C radioactivity among acidic (black bars, organic acids), neutral (hatched bars, sugars), and basic (white bars, amino acids) fractions after ion-exchange chromatography of *L. albus* root and leaf extracts. Plants were grown with either 1 mM P (+P-treated) or without P (−P-treated) for 14 DAE and shoots and roots were labeled independently with $^{14}CO_2$. Shoot-labeled plants were exposed to 0.37 MBq and root-labeled plants were exposed to 3.7 MBq $^{14}CO_2$ for 60 min. After the 22-h chase, normal roots, proteoid roots, and leaves (youngest, fully expanded) were harvested and extracted with 50% ethanol. Each bar represents the treatment mean, $n = 5$.

Table II. *Flux densities (μmol h^{-1} g^{-1}), total flux (in 22 h) (μmol) and specific radioactivity (Bq μmol^{-1}) of citrate and malate recovered in root exudates*

Plants were grown with either 1 mM P (+P treated) or without P (−P treated) for 14 DAE and shoots and roots were labeled independently with $^{14}CO_2$. Shoot-labeled plants were exposed to 0.37 MBq and roots to 3.7 MBq $^{14}CO_2$ for a 60-min pulse period followed by a 22-h chase. Exudates were collected as eluted nutrient solution at 3 and 22 h into the chase (mean ± SD, n = 4).

$^{14}CO_2$ Labeling Site	Treatment	Root Weight (g)	Flux Densities		Total Flux		Specific Radioactivity	
			Citrate	Malate	Citrate	Malate	Citrate	Malate
			μmol h^{-1} g^{-1}		μmol		Bq μmol^{-1}	
Root	+P	7.0	0.02	0.03	3	4	ND[a]	ND
Root	−P	5.5	0.57	0.51	70	60	240 ± 106	96 ± 28
Shoot	+P	7.0	0.05	0.04	7	3	ND	ND
Shoot	−P	5.2	0.97	0.66	103	60	87 ± 13	39 ± 17
Significance of labeling site		NS[b]	NS	NS	NS	NS		
Significance of treatment		**[c]	**	**	**	**		
LSD for interactions (α = 0.05)		NA[d]	NA	NA	NA	NA		

[a] ND, Not detected. [b] NS, Not significant. [c] **, Significant at P = 0.01 level. [d] NA, Not applicable; interaction term was not significant.

Concentration and Distribution of Free Amino Acids in Plants

Asn, Gln, Ser, Ala, Pro, and Val represented 82 to 94% of the free amino acids detected by HPLC (Table IV). Roots from the −P-treated plants had 2.5- to 3.4-fold more free amino acids than roots from the +P-treated plants. This increase in free amino acids in the −P-treated normal and proteoid roots was attributable to a 3.4- to 5.3-fold increase in Asn concentration compared with +P-treated normal and proteoid roots. The concentrations of Arg and Leu also were increased in −P-treated roots compared with +P-treated roots, but these amino acids represented only a small percentage of the total pool of free amino acids (Table IV). The concentrations of the remaining free amino acids were not altered by P treatment. The radioactivity in individual amino acids after HPLC was below the detection limit of the LSC, so specific radioactivity is not reported.

In Vitro Specific Activity of PEPC

In vitro specific activity of PEPC was 2- to 7-fold higher in −P-treated proteoid roots than in any other treatment combination (Fig. 3). There was a tendency for the +P-treated proteoid roots to have a higher in vitro specific activity than +P-treated normal roots, which is consistent with our prior results (Johnson et al., 1994) showing that proteoid roots have a higher average in vitro specific activity than normal roots from both P treatments. The protein concentration averaged 1.7 mg g^{-1} fresh weight of root and 16.5 mg g^{-1} fresh weight of leaves.

CO_2 Fixation and Recovery of C in Root Exudates

The in vivo CO_2 fixation rate in −P-treated roots was 4-fold higher than in +P-treated roots (Table V). The rate of C fixation was 12-fold higher in shoots than in roots when averaged over P treatment. However, shoot CO_2 fixation rate was not different between P treatments. Acid-stable radioactivity was used to calculate CO_2 fixation rates, and does not include respired $^{14}CO_2$; therefore, estimated fixation rates may underestimate the actual fixation rates.

The −P-treated plants exuded 14-fold (shoot-labeled) to 24-fold (root-labeled) more total C than +P-treated plants (Table V). Comparable differences in exudation were ob-

Table III. *Concentration (μmol g^{-1} fresh wt) and specific radioactivity (Bq μmol^{-1}) of dominant organic acids in L. albus roots or leaves*

Plants were grown with either 1 mM P (+P-treated) or without P (−P-treated) for 14 DAE and shoots and roots were labeled independently with $^{14}CO_2$. Shoot-labeled plants were exposed to 0.37 MBq and roots to 3.7 MBq $^{14}CO_2$ for a 60-min pulse period. After the 22-h chase, normal roots, proteoid roots, and leaves (youngest fully expanded leaf) were harvested and extracted with 50% ethanol. Extracts were separated by ion-exchange chromatography and the organic acid fraction was separated by HPLC. Values are averaged over $^{14}CO_2$ labeling site (n = 10).

Treatment	Organ	Concentration			Specific Radioactivity		
		Citrate	Malate	Succinate	Citrate	Malate	Succinate
		μmol g^{-1} fresh weight			Bq μmol^{-1}		
+P	Normal roots	1.4	11.0	9.0	30.6	24.0	15.8
+P	Proteoid roots	3.3	13.4	7.7	50.3	27.7	19.0
+P	Leaves	3.1	37.9	35.7	26.2	17.4	13.5
−P	Normal roots	4.1	12.3	8.7	58.1	22.9	16.7
−P	Proteoid roots	4.9	9.6	4.1	53.7	23.0	17.2
−P	Leaves	2.8	31.3	25.8	29.1	18.6	11.6
Significance of treatment		**[a]	NS[b]	**	NS	NS	NS
Significance of organ		**	**	**	**	**	NS
LSD for interaction (α = 0.05)		1.1	NA[c]	3.9	20.3	NA	NA

[a] **, Significant at P = 0.01 level. [b] NS, Not significant. [c] NA, Not applicable; interaction term was not significant.

Table IV. *Concentration (μmol g^{-1} fresh wt) of dominant amino acids in L. albus roots and leaves*

Plants were grown with either 1 mM P (+P-treated) or without P (−P-treated) for 14 DAE and shoots and roots were labeled independently with $^{14}CO_2$. Shoot-labeled plants were exposed to 0.37 MBq and roots to 3.7 MBq $^{14}CO_2$ for a 60-min pulse period. After the 22-h chase, normal roots, proteoid roots, and leaves (youngest fully expanded leaf) were harvested and extracted with 50% ethanol. Amino acids were derivitized with dabsyl-Cl and separated by HPLC. Means are averaged across the site of labeling (n = 6).

Treatment	Organ	Asn	Gln	Glu	Ser	Ala	Pro	Val	Arg	Leu	Total
						μmol g^{-1} fresh wt					
+P	Normal roots	7.77	1.18	0.40	1.19	1.18	0.90	0.92	0.17	0.06	14.4
+P	Proteoid roots	5.32	1.25	0.35	1.01	0.71	2.14	0.35	0.20	0.20	11.4
+P	Leaves	1.94	0.60	0.46	1.50	0.87	3.64	0.41	0.20	0.17	11.0
−P	Normal roots	25.19	1.00	0.30	1.22	0.98	2.73	0.85	0.22	0.52	35.4
−P	Proteoid roots	28.43	1.38	0.24	0.89	0.50	2.87	0.54	0.49	0.57	38.3
−P	Leaves	6.62	1.16	0.28	0.60	0.38	1.50	0.26	0.14	0.42	13.1
Significance of treatment		**[a]	NS[b]	NS	NS	NS	NS	NS	**	**	*[c]
Significance of organ		**	NS	NS	NS	*	NS	*	*	NS	**
LSD for interaction (α = 0.05)		12.19	NA[d]	NA	0.80	NS	NA	NS	0.13	NA	17.8

[a] **, Significant at P = 0.01. [b] NS, Not significant. [c] *, Significant at P = 0.05. [d] NA, Not applicable; interaction term was not significant.

served when exudation was expressed in terms of radioactivity (Table I). C recovered in citrate and malate accounted for 63 to 87%, respectively, of the C exuded in the crude exudate from −P-treated plants. The remaining C was from sugars and amino acids or was lost during analysis (Fig. 1).

Contribution of Nonphotosynthetic C Fixation in Roots to Exuded C

C fixed in roots contributed 32% of the C exuded from −P-treated plants, but only 17% of the C exuded from +P-treated plants (Table VI). These values were significantly different at P = 0.03, despite the large SD (Table VI).

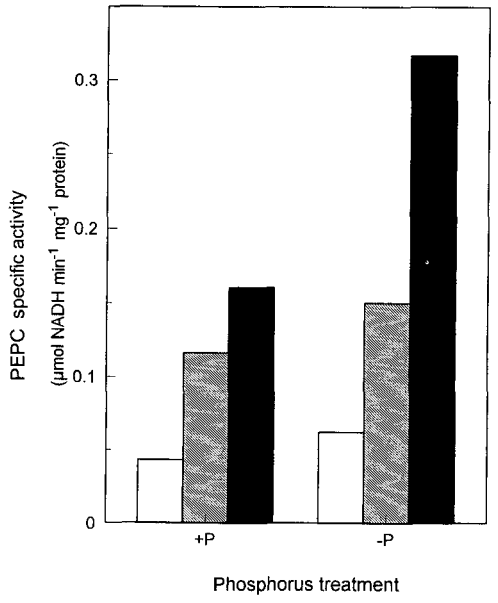

Figure 3. In vitro specific activity of PEPC (μmol NADH min^{-1} mg^{-1} protein) determined spectrophotometrically at A_{340} for leaves (white bars), normal roots (hatched bars), and proteoid roots (black bars) of *L. albus* plants grown with either 1 mM P (+P-treated) or without P (−P-treated) for 14 DAE. Each bar represents the treatment mean (n = 6). $LSD_{\alpha=0.5}$ = 0.09 for all interactions.

C fixed in the roots of −P-treated plants contributed 25 and 34% of the C exuded as citrate and malate, respectively (Table VI). A similar contribution (25–30%) of C fixed in alfalfa nodules via PEPC for N assimilation was reported by Vance et al. (1983) and Maxwell et al. (1984).

DISCUSSION

Root Exudates

P-deficient white lupin roots exude striking amounts of citrate and malate (Table II). These metabolites have a high specific radioactivity, particularly when the roots are the site of in vivo labeling. Although exudation of citrate by P-deficient white lupin has been reported previously (Gardner et al., 1983; Dinkelaker et al., 1989; Braum, 1995), and an increase in the concentration and specific radioactivity of malate and citrate exuded by P-deficient rape relative to P-sufficient plants was reported by Hoffland et al. (1992), to our knowledge, our study is the first to show that a substantial proportion of the C exuded by P-deficient lupin is derived from nonphotosynthetic C fixation in roots (Tables V and VI).

In −P-treated plants 32 to 66% of the acid-stable radioactivity was recovered in exudates (Table I), compared with 3 to 5% in the +P-treated plants. The amount exuded by the +P-treated plants is comparable to exudation of nonvolatile ^{14}C (2–5%) by nonstressed, shoot-labeled rape (Shephard and Davies, 1993). Rhizodeposition of water-soluble metabolites, secretion, and lysate can represent 10 to 30% of net radioactivity fixed by pea (*Pisum sativum* L.) and tomato shoots (Whipps, 1987).

In the present study, over 90% of the radioactivity recovered in root exudates from −P-treated plants was in the organic acid fraction (Fig. 2) and, of that, most (75%) was in citrate and malate (Table V). Exudation of organic acids, especially citrate, is a mechanism to increase the availability of P (Gardner et al., 1982, 1983; Dinkelaker et al., 1989; Hoffland, 1992; Gerke et al., 1994; Jones and Darrah, 1994; Braum, 1995). Exudation of organic acids in response to nutrient deficiency, particularly P and Fe, is well docu-

Table V. *C fixation rates (nmol C min^{-1} g^{-1} fresh wt) and recovery of fixed C (μmol) in root exudates from L. albus*

Plants were grown with either 1 mM P (+P-treated) or without P (−P-treated) for 14 DAE and shoots and roots were labeled independently with $^{14}CO_2$. Shoot-labeled plants were exposed to 0.37 MBq and roots to 3.7 MBq $^{14}CO_2$ for a 60-min pulse period. C fixation rate was calculated from Bq fixed during the 60-min pulse. Calculation of C recovery was based on acid-stable radioactivity in exudates and the specific radioactivity (Bq $nmol^{-1}$ CO_2) of C in the labeling chamber. See sample calculations 1 to 4. Mean ± SD, n = 5, except recovery of C in citrate and malate, n = 4.

$^{14}CO_2$ Labeling Site	Treatment	Organ Weight (g)	C Fixation Rate	Recovery of C		
				Total root exudate	Citrate	Malate
			nmol C min^{-1} g^{-1} fresh wt		*μmol C*	
Root	+P	7.0	2.6 ± 0.5	0.1 ± 0.1	ND[a]	ND
Root	−P	5.5	11.1 ± 1.7	2.4 ± 0.3	1.0 ± 0.6	0.5 ± 0.3
Shoot	+P	3.4	72.3 ± 24.7	0.4 ± 0.3	ND	ND
Shoot	−P	2.9	102.0 ± 36.1	5.5 ± 1.9	3.5 ± 2.0	1.3 ± 1.3
Significance of labeling site		**[b]	**	*[c]		
Significance of treatment		*	*	**		

[a] ND, Not detected. [b] **, Significant at P = 0.01. [c] *, Significant at P = 0.05.

mented (Landsberg, 1981; Gardner et al., 1983; Marschner et al., 1986; Lipton et al., 1987; Dinkelaker et al., 1989; Hoffland et al., 1992). Citrate exudation by white lupin roots is particularly important since this species has no mycorrhizal associations (Gardner et al., 1982; Avio et al., 1990). Exudation of sugars and amino acids can be enhanced by P stress and has been suggested as a mechanism to enhance mycorrhizal association (Ratnayake et al., 1978; Graham et al., 1981). Although proteinaceous amino acids are released into the rhizosphere, they have a limited role in the direct acquisition of nutrients by plants (Jones et al., 1994). Only a small amount of radioactivity (<10%) is recovered as amino acids from the rhizosphere (Fig. 1), and in preliminary HPLC studies we were unable to resolve individual amino acids in exudates. These results are consistent with the results of Jones and Darrah (1993), who showed that up to 90% of amino acids passively diffused to the rhizosphere can be resorbed by roots.

We found that −P-treated white lupin exuded 3.3% of its dry weight as citrate by 14 DAE (Table III) and exuded 5.6% by 22 DAE (Johnson et al., 1996). These values are similar to the quantity of citrate exuded by P-deficient white lupin after 6 weeks (6–11%) (Gardner et al., 1983; Braum, 1995) and after 13 weeks (23%) (Dinkelaker et al., 1989). In a soil environment, as much as one-third of the citrate may be adsorbed rapidly (Braum, 1995). In a nonsterile environment, the amount of citrate measured in exudates would represent the concentration maintained against microbial degradation (Braum, 1995). Even in sand culture the reported amounts of citrate in root exudates may underestimate the total amount of C exuded due to degradation. The amount of citrate exuded by white lupin is remarkable, especially when compared with alfalfa, which releases only 0.3% of its dry weight when it is P deficient (Lipton et al., 1987).

Nearly 25-fold more citrate per unit root was recovered in root exudates from −P-treated plants compared with from +P-treated plants (Table II). In addition, 2.8-fold more citrate was recovered in root exudates of −P-treated plants in 22 h than the total amount of citrate in the entire plant (30 μmol) and 4.5-fold more than the amount of citrate in the root system (Tables II and III). In comparison, the amount of malate recovered in root exudates from −P-treated plants in 22 h reflected about 40% of the total amount of malate detected in −P-treated plants (153 μmol) (Tables II and III). Succinate, although not detectable in the exudate after 22 h, represented 22 to 34% of the total organic acid pool in roots of −P-treated plants. Presumably, if organic acids were passively leaking or diffusing across membranes, the relative composition of exudates would reflect the composition of the tissue. Conclusions about active or passive flux of organic acids require knowledge of compartmentalization of metabolites and relevant membrane potentials. Lacking this information, our data are consistent with the hypothesis that active excretion of both protons and citrate appears necessary to achieve efficient P mobilization (Dinkelaker et al., 1989; Jones and Darrah, 1994). Electrochemical gradients across root plasmalemma suggest that citrate is excreted via an anion channel with protons supplied by plasmalemma ATPases under stress conditions (Jones and Darrah, 1994). However,

Table VI. *Contribution of C derived from nonphotosynthetic C fixation in roots to C recovered in root exudates*

Plants were grown with either 1 mM P (+P-treated) or without P (−P-treated) for 14 DAE and shoots and roots were labeled independently with $^{14}CO_2$. Shoot-labeled plants were exposed to 0.37 MBq and roots to 3.7 MBq $^{14}CO_2$ for a 60-min pulse period. C fixation rate was calculated from Bq fixed during the 60-min pulse. Calculation of C recovery was based on acid-stable radioactivity in exudates and the specific radioactivity (Bq $nmol^{-1}$ CO_2) of C in the labeling chamber. See sample calculations 4 and 5 (mean ± SD).

Treatment	Total Root Exudate (%)	Root Exuded Citrate (%)	Root Exuded Malate (%)
+P	17.2 ± 17.0	ND[a]	ND
−P	32.1 ± 10.5	24.8 ± 8.5	34.4 ± 12.6
Number of observations	5	4	4

[a] ND, Not detected.

citrate may be exuded by passive diffusion under nonstress conditions (Jones and Darrah, 1994).

Free Amino Acids

The mechanism by which free amino acids, particularly Asn, are increased in P-stressed white lupin is not known (Table IV); several mechanisms are possible. Protein biosynthesis may be inhibited in −P-treated plants or protein degradation may be enhanced. Increases in free amino acid concentration due to the inhibition of protein synthesis have been associated with other mineral nutrient deficiencies (K, Mg, and Zn [Marschner, 1995]). In P-deficient tobacco (*Nicotiana tabacum* L.) elevated concentrations of amino acids were associated with degradation of protein rather than inhibition of protein synthesis (Rufty et al., 1990). The pattern of amino acid translocation also may be altered; translocation of Asn is reduced drastically by P deficiency in soybean (Rufty et al., 1993). P deprivation alters protein synthesis profiles of *Brassica nigra* suspension cells (Fife et al., 1990). The accumulation of specific amino acids could be altered by increased biosynthesis or reduced incorporation of specific amino acids into proteins.

Origin of Exuded C

When plants are shoot-labeled, over 70% of the radioactivity is in the neutral fraction (sugars) of extracts of normal and proteoid roots (Fig. 2). However, most of the radioactivity in root exudates from these plants is in organic acids (Fig. 1), predominantly citrate and malate (Table II). These data indicate that ^{14}C fixed via C_3 photosynthesis in the shoots is translocated to the roots primarily as sugars, but is converted to citrate and malate in the roots before exudation. This is consistent with the pathway suggested for malate exudation in P-deficient rape (Hoffland et al., 1992). Malate rather than pyruvate may be the terminal product of glycolysis and the primary substrate of the TCA cycle in plants (Lance and Rustin, 1984).

The fact that 66% of the total ^{14}C fixed by root-labeled, −P-treated plants is exuded (Table I) suggests that under P-stress conditions, C fixed in roots plays an important role in C exudation. To our knowledge, this is the first report quantifying the amount of ^{14}C exuded by root-labeled plants. In roots, C is fixed primarily by PEPC (Christeller et al., 1977; Maxwell et al., 1984; Landsberg, 1986; Vuorinen et al., 1992; Johnson et al., 1994). The in vitro specific activity of PEPC in −P-treated proteoid roots is 2- to 6-fold higher compared with all other organs after either P treatment (Fig. 3). The increase in C fixation in −P-treated roots is likely due to a combination of mechanisms, including an increase in PEPC activity (Fig. 3), an increase in PEPC protein, and an increase in proteoid roots (Johnson et al., 1994). Although the vast majority of C economy studies recently reviewed by Whipps (1990) determined only the fate of C fixed in shoots and did not consider nonphotosynthetic C fixed in roots, our data suggest that the latter may play an important role in C balance of P-deficient plants.

The C fixation rate of −P-treated roots from white lupin, a C_3 plant, is enhanced more than 4-fold compared with +P-treated roots. The C fixation of the −P-treated roots is comparable to that of NO_3^--fed maize roots (12.5 nmol C min^{-1} g^{-1} fresh weight), a C_4 species (Cramer et al., 1993). The rate of C fixation in the +P-treated roots is comparable to rates reported for nodulated alfalfa roots (0.3–2.2 nmol C min^{-1} g^{-1} fresh weight) (Maxwell et al., 1984). By contrast, nodulated soybean (*Glycine max* L. Merr) roots and NH_4^+-fed maize roots average much higher rates of C fixation (33 and 75 nmol C min^{-1} g^{-1} fresh weight, respectively) (Coker and Schubert, 1981; Cramer et al., 1993).

Our results suggest that citrate exuded from proteoid roots is synthesized, in part, from C fixed via PEPC in those roots. This is based on the increased specific activity of PEPC (Fig. 3), MDH (EC 1.1.1.37), and CS (EC 4.1.3.7) (Johnson et al., 1994) in proteoid roots compared with normal roots, and on the fact that nonphotosynthetic C fixed in excised proteoid roots (Johnson et al., 1994) as well as in intact roots is rapidly incorporated into citrate and malate (Tables II and III). In addition, Marschner et al. (1987) demonstrated that proteoid roots are the specific site of citrate exudation in P-deficient lupin.

The predicted contribution of nonphotosynthetic C fixation to citrate and malate exudation is 16.6 and 25%, respectively. This prediction assumes that one C atom per molecule of exuded citrate or malate originated from nonphotosynthetic C fixation via PEPC in the roots. Observed values were 1.5-fold more than predicted (Table VI), suggesting that about half of the citrate and malate exuded was double labeled. C fixed via PEPC would initially be incorporated into OAA by the carboxylation of PEP (Goodwin and Mercer, 1983; Rawn, 1989). Subsequently, OAA could be condensed with acetyl-CoA to form citrate by CS. Citrate exuded at this point would have a single label. The OAA could also be converted by MDH to malate. The activities of CS and MDH are higher in proteoid roots compared with normal roots (Johnson et al., 1994). Malate is in equilibrium with fumarate and during this interconversion reaction, the relative position of the label can be shifted between C-1 and C-4. Subsequently, malate could (a) be exuded with a single label; (b) provide C for citrate synthesis in the TCA cycle (label in the C-4 position would not be removed during decarboxylating steps in the TCA cycle and could go through more than one turn of the TCA cycle, resulting in double-labeled organic acids); and/or (c) be decarboxylated by malic enzyme to form pyruvate. Label in pyruvate could subsequently label acetyl-CoA or PEP, and either route could result in double-labeled citrate or malate. It is not possible to determine the precise pathway of the ^{14}C label from our data, since there are multiple pathways that could result in a double label on exuded citrate and malate.

CONCLUSIONS

The results of this research confirm earlier findings that P deficiency greatly enhances the exudation of the organic acids citrate and malate, and modifies amino acid biosynthesis, transport, or protein synthesis. It dem-

onstrates that nonphotosynthetic C fixation provides from one-fourth to one-third of the C required for the synthesis of citrate and malate that is exuded from P-deficient lupin roots. Nonphotosynthetic C fixation in the roots of white lupin is an integral component of the P acquisition strategy that results in the exudation of large amounts of citrate and other organic acids that increase P availability in the rhizosphere.

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